REMARKS

Claims 1-9, 20-22 have been examined. Two claims were objected to. All pending claims were rejected under § 102. Applicants have carefully studied the Office Action and respond thereto. Claim 1 and withdrawn claim 11 have been amended to indicate that the cells being transduced with IL-10 are not selected on the basis of specificity for a predetermined antigen. Claim 22 is canceled without prejudice or disclaimer.

A number of amendments have been made voluntarily to clarify and otherwise improve the claim language. These amendments, not in response to any matters raised by the Office, include:

In the Active Claims	
1	Removal of the "providing" step (a) and introduction of the necessary language from it into step (b) which becomes step (a) in the amended claim, along with renumbering step (c) as (b)
	Amended step (a) also includes other minor changes to improve clarity, such replacement of "introducing" with "modifying" at least a portion of the cell "population...by introducing..."
	Addition of a "closing" clause that reads: "thereby producing said population of mononuclear cells overexpressing IL-10 ..."
	(Dependent claims that recite the step numbers are appropriately amended)
2	replacement of "wherein and "enriched fraction...of cells...are provided" with "wherein the expression construct is introduced into an enriched fraction...of said...cell population"
3	the word "subset" is moved
9	"modified" is added before "mononuclear cells and "IL-10-encoding" is added before "nucleotide sequence", all for greater clarity
20/21	"cells" is amended to "lymphocytes" as a more precise term, without changing any meaning; "enriched" is added before "lymphocyte subset" for greater clarity

In Withdrawn Claims	
12	"T lymphocyte" is added before "composition", which is followed by "that is a fraction of the mononuclear cells..."
13	"T lymphocyte" is added before "composition"

Support for the amendments related to "antigen specificity" in claims 1 and 11 can be found throughout the specification, though in particular at page 17, lines 5-7. Other amendments are supported by the original claims and the specification throughout. These amendments do not introduce new matter and their entry is respectfully requested.

A discussion of the Office Action and Applicants' responses to various grounds of objection and rejection follow.

I. Claim Objections

A. Claim 10

Claim 10 was objected to because there were *two copies of claim 10* in the paper of 4/26/06. Applicants inadvertently included an earlier version of claim 10 along with the (intended) newly amended version, as follows:

> 10. *(previously presented):* A method for producing a pharmaceutical composition comprising mononuclear cells overexpressing IL-10, which method comprises combining
> (a) the mononuclear cells overexpressing IL-10 produced in accordance with claim 1 with
> (b) an acceptable pharmaceutical carrier.
>
> 10. ***(currently amended):*** A method for producing a pharmaceutical composition comprising mononuclear cells overexpressing IL-10, which method comprises ~~combining~~
> (a) producing the mononuclear cells overexpressing IL-10 ~~produced~~ in accordance with claim 1, and ~~with~~
> (b) combining said cells with an acceptable pharmaceutical carrier.

Applicants hereby cancel the earlier version of claim 10 above , and retain, the second version, which the Examiner withdrew from consideration in refusing Applicants request that this claim be joined with the elected claims. Thus, claim 10 presently appears in the Listing of Claims above with the designator *"withdrawn; previously presented"* as it had been amended once. This objection may now be withdrawn.

B. Claim 22

Claim 22 was objected to as being of improper dependent form for failing to further limit a previous claim. Claim 22 is being cancelled as shown in the *Listing of Claims*, thereby obviating this objection.

II. REJECTIONS UNDER 35 U.S.C. § 102

Three separate anticipation rejections were applied to various claims under § 102, and are discussed separately below

A. First Rejection under § 102(b)

Claims 1-5, 9, 20 and 22[1] were as being anticipated by **Moritani *et al.,*** 1996, *J.Clin Invest. 98*:1851-1859 (hereinafter "Moritani"). Claims 6-8 appeared to be free of this rejection.

1. The Office's Discussion of Moritani

Moritani allegedly describes the isolation of Th1 peripheral blood mononuclear cells (PBMC) from mice, the isolation of single cell clones, culture of the Th1 cells with antigen ("Ag") and irradiated Ag- presenting cells (APCs) to stimulate their proliferation, and the

transfection of such cells with a construct encoding IL-10 and a *neo* cassette. According to the Office, Thl cells are inherently CD4+ T cells. The Action goes on to state that Moritani teaches enrichment in culture of the transfected population, to select for Th1 cells expressing IL-10. By gradually increasing the concentration of the selective agent (the antibiotic G418), Moritani is said to be selecting for cells that overexpress *neo*, and, consequently, IL-10. Finally, Moritani is said to teach that the enriched Thl cells are transferred into NOD mice. The Office concluded from the foregoing that all the limitations of the pending claims were found in Moritani, thereby anticipating the indicated claims.

2. Applicants' Response

Applicants have amended claims 1 and 11 explicitly (and thereby, all the claims dependent thereon), to require that the cells being transduced with IL-10 (and as a result, the transduced cells produced by the claimed method) are not selected on the basis of their specificity for a predetermined Ag. In other words, all the present claims are directed to a method in which an IL-10 nucleic acid is used to modify (A) PBMCs or (B) a fraction or subset of cells present in the PBMC population such as

(1) lymphocytes such as T or B cells, or their subsets -- primarily CD4+ T lymphocytes
(2) monocytes;
(3) macrophages; or
(4) dendritic cells DCs).

Importantly, even when subsets are enriched or selected, the selection is **not** on the basis of Ag-reactivity or Ag-specificity. Moreover, regardless of whether the cells are induced to proliferate or differentiate *before* transduction (*e.g.*, claims 4-6) , or are fractionated or enriched *before* (claims 2 and 3) or *after* (claims 7-9) transduction, in no case is such stimulation, fractionation or enrichment carried out using a specific Ag or based on the cells' Ag-specificity.

This is in contrast with Moritani - where the process **begins** with an antigen-specific selection process. First, the Moritani studies are all conducted on "islet-specific" (=Ag-specific) clones of Th1 CD4+ T cells (pages 1851-1852). Second, these clones were stimulated to grow by culture with irradiated dispersed NOD islet cells "as Ag," plus a source of "Ag-presenting" cells (APCs). There could not be a more stringent and unambiguous selection process than that of isolating and propagating clones of T cells that are specific for an Ag. It is these cells that are then transduced with IL-10 and used for therapy of genetically diabetic (Type I diabetes) recipient mice in an antigen-specific manner.

[1] In view of the cancellation of claim 22, this claim is not listed in describing the subsequent art rejections.

Clearly, then, the presently method is distinct from *any* method in which the cells being transduced with IL-10 (and subsequently used therapeutically) are selected from the outset to be Ag-specific and are expanded in an Ag-specific manner such as disclosed in Moritani as well as in the other cited references (discussed below). For this reason, it would be proper to withdraw the § 102 rejection based on Moritani.

B. Second Rejection under § 102(b)

Claims 1-8, 20 and 21 are rejected under 35 U.S.C. 102(b) as anticipated by Setoguchi *et al., J. Immunol. 165*:5980-5986) (hereinafter "Setoguchi"). Claim 9 appears to be free of this rejection.

1. The Office's Discussion of Setoguchi

Setoguchi was said to describe a method of isolating splenocytes from mice immunized with the Ag ovalbumin (OVA), proliferating these cells for 48 hours in the presence of OVA (to obtain OVA-specific splenocytes) or PHA (to obtain Ag-nonspecific splenocytes). These cells were transfected with a vector encoding IL-10 (pg. 5981, Materials and Methods; pg. 5983, col. 1). Further, Setoguchi is said to teach enriching CD4+ T cells from among these (**Ag-specific**) splenocytes post transfection (pg. 5981, co1.2) and transferring them into mice (pg. 5981, col. 2). On this basis, the Office concluded that Setoguchi teaches all the limitations of the indicated claims.

2. Applicants' Response

First, the specification discusses Setoguchi in some detail at page 2, line 31 to page 3, line 8. The Applicants' concluded from the results of the Setoguchi study that:

> ...this use of IL-10 transduced T cells is strictly limited to T cells that are specific for a predetermined antigen. As for most (if not all) inflammatory diseases the relevant antigens are not known, this antigen-specific application of IL-10-expressing T cells currently has no practical therapeutic value.

This statement focuses one on the novelty of the present invention as currently claimed and its fundamental difference from Setoguchi. The present invention, as reflected in claim 1, is directed to a method for producing IL-10-transduced and expressing PBMCs that have not been pre-selected on the basis of Ag-specificity, and further, that the cells are for treating an inflammatory disease in an antigen-independent manner and that the cells are capable of mediating such treatment. This is in stark contrast with the three cited references.

The Office Action's reference to the IL-10 transduction of "Ag-nonspecific (T) cells" must be viewed in the context of the actual experiments reported. The Office will presumably

admit that the only "active" and "useful" CD4+ T cells in Setoguchi are derived from spleen cells of D011.10 transgenic (Tg) mice. These cells are highly Ag-specific because they carry a transgene that encodes a T cell receptor that is specific for OVA (see section on "Mice" under *Materials and Methods* (pg. 5981, left col.). The whole purpose of using the D011.10 mouse strain was to start out with a homogeneously Ag-specific cell population.

The language to which the Action refers is found in a section titled *Infection of the Retrovirus* at page 5981, right col.). Careful review of this paragraph indicates that as controls for Ag-specific cells infected with a retrovirus carrying the IL-10 gene and stimulated with OVA, "Ag nonspecific splenocytes" (not peripheral blood mononuclear cells) were infected with the retrovirus in the presence of a polyclonal activator (mitogen) PHA instead of OVA. This use of mitogen vs. antigen was only because of the well-known requirement that cells be actively proliferating for successful retrovirus infection (and transduction with the retrovirus vector-borne IL-10 gene). Applicants believe that it is somewhat ambiguous for the Office to characterize this retroviral infection process simply as "proliferating these cells for 48 hours in the presence of OVA (to obtain OVA-specific splenocytes) or PHA (to obtain Ag- non-specific splenocytes)."

Now what were these control cells used for? The only place in the paper it appears their use was discussed is that described in the Results section, in a subsection entitled *Amelioration of the disease severity requires Ag-specificity* (page 5983, left col.). The reference states: "...[W]e transferred wild-type Balb/c splenocytes transfected with IL-10" into arthritic recipients. These splenocytes are presumably the cells referred to in the *Materials & Methods* section discussed above. - though that paragraph does not explicitly say that Ag-nonspecific cells were from spleens of Balb/c mice. Importantly, mice receiving these "control" IL-10 transduced cells developed Ag-induced arthritis as severe as that of *positive controls* (in whom arthritis was induced but no therapeutic cells were given). In contrast, the Ag-specific transduced splenocytes (from DO11.10 mice) caused amelioration of the disease symptoms. This led the authors to conclude that Ag-specificity of the CD4+ T cells was " **indispensable** for efficient reduction of disease severity..."

One is forced to conclude from this study that non-Ag-specific "control" lymphocytes which are presumably the basis for the rejection, have **no discernible activity or utility** other than serving as a negative control for this particular study! Indeed, the language quoted from the specification above shows that Applicants "recommend" a totally different approach for their invention, as set out in the present claims. Indeed, the present invention is intended to provide IL-10-producing regulatory T cells for use in the therapy of inflammatory diseases "in an

antigen-independent manner," as well as providing methods of obtaining (producing) such regulatory T cells (specification at page 3, lines 15-16).

Based on the foregoing, the difference between the present claims and the Setoguchi reference should be obvious, so that the rejection may properly be withdrawn.

C. Third Rejection under 35 U.S.C. 102(e)

Claims 1-6 and 20 were rejected under 35 U.S.C. 102(e) as being anticipated by US Pat. Pub. 2001/0033836 Al published (10.25.2001) having a priority date of 9.19.1997, hereinafter "Symonds". Claims 7-9 and 21 appear to be free of this rejection.

1. The Office's Discussion of Symonds

The Action indicates that Symonds shows the isolation of CD4+ cells from the peripheral blood of an individual with rheumatoid arthritis (RA) (paragraphs 21, 59, Claim 1). The cells are enriched for CD4+ cells and cultured with chicken or bovine collagen type II or autologous synovial fluid in the presence of antigen presenting cells or an anti-CD3 mAb. CD4+ T cells reactive to collagen type II or antigens within the synovial fluid were then expanded with IL-2. The IL-2 gene was introduced into these cells using a retroviral vector, the transduced cells expanded and injected into patients. The Office asserts that "these cells are CD4+ but are **not selected for a specific antigen**" and concludes that all the limitations of the claims are present in Symonds.

2. Applicants' Response

Applicants respectfully disagree with the Office's interpretation of this reference and cannot understand on what basis the Examiner concluded that the disclosed cells and methods are not based on selection for a specific Ag.

As noted in Para [0021] of Symonds, the disclosed method comprises isolating $CD4^+$ cells from a subject who has an Ag-specific disease (defined in Symonds as "a disorder characterized by the presence of a unique epitopic locus"). What is the "epitopic locus" if not "an antigen"? These isolated $CD4^+$ cells are treated "so as to enrich the population of cells therein which specifically bind to the unique epitopic locus." To Applicants this can only mean enriching Ag-binding (= Ag-specific) CD4+ cells. These cells are then transduced with a "therapeutic protein" ("TP"), such as IL-10 which is said to be "capable of ameliorating the effects of the disorder". This TP / IL-10 is expressed and secreted from such cells. Finally, such TP- (read IL-10-) transduced cells are administered to the subject to treat the disorder.

Symonds defines "unique epitopic locus" at para. [0035] as

> ...the surface area on a single **antigenic** molecule, or formed by a plurality of **antigenic** molecules, which (a) exists at or near the location of the disorder in the afflicted subject, (b) does not exist at or near this site in an unafflicted subject, and (c) can be **recognized by and specifically bound to $CD4^+$ cells** present in the afflicted subject. ... For example, a unique epitopic locus can be the surface area of exposed and degraded **collagen type II** at and near the inflamed joint of a subject afflicted with **arthritis**...

(emphasis added).

Paragraph [0036] provides examples of disorders and their corresponding unique epitopic loci (*i.e.*, Ages):

> ...rheumatoid arthritis (collagen type II, synovial material); multiple sclerosis (myelin basic protein, proteolipid protein, myelin oligodendrocyte glycoprotein); myasthenia gravis (nicotinic acetylcholine receptor); insulin-dependent diabetes mellitus (proinsulin, glutamic acid decarboxylase); primary biliary cirrhosis (nuclear pore complex proteins, 2-oxo-acid dehydrogenase); psoriasis (cutaneous leukocyte antigen, Streptococcal M protein); Crohn's disease ($\alpha 4\beta 7$ integrin); Guillain-Barre syndrome (peripheral myelin protein); autoimmune gastritis (gastric H^+/K^+ ATPase, intrinsic factor); Addison's disease (21-hydroxylase); Graves' disease (thyroid stimulating hormone receptor); Hashimoto's thyroiditis (thyroid peroxidase); and autoimmune uveitis (S-antigen).

This is a "classic" list of autoantigens associated with various autoimmune/inflammatory diseases.

At paragraph [0040] Symonds defines enrichment of $CD4^+$ cells in terms of the percentage of cells that "specifically bind to a unique epitopic locus" (*i.e.*, Ag) "after treatment" (*i.e.*, enrichment) compared to controls as being at least 2-fold, preferably at least 10-fold. Clearly, Symonds is talking about enriching Ag-specific cells.

The Examples support Applicants' interpretation. Example 1, paragraphs [0058]- [0060] is directed to RA. Here $CD4^+$ cells isolated from peripheral blood are cultured in the presence of an Ag (chicken or bovine collagen type II or autologous synovial fluid) plus APCs. **Ag-reactive cells that have been so selected** are next expanded nonspecifically wit IL-2. Only after this Ag-based selection process is the gene encoding an anti-inflammatory cytokine such as IL-10 transduced into the CD4+ cells.

Paragraph [0060] refers to therapy with "cells with T-cell receptor specificity for collagen type II or other joint or synovial proteins". This is another way of saying "Ag-specific cells."

Example 2 (paragraphs [0061] - [0063] describe a murine arthritis model, that of collagen-induced (=Ag-induced) arthritis. Symonds discloses manipulating $CD4^+$ cells *in vitro* in the presence of autologous APCs and collagen type II (Ag) , to induce proliferation of cells with known CD4+ cell receptor (*i.e.*, Ag-) specificity. This is a step of **selecting Ag-specific cells**. Thereafter, the collagen-reactive (Ag-reactive) $CD4^+$ cells are engineered to express an anti-inflammatory protein, expanded, and re-injected into mice with collagen-induced arthritis.

There is no question that Symonds indeed discloses Ag-based selection of Ag-specific cells for subsequent transduction with IL-10. Again, this is distinct from the present claims. Given this difference, Symonds cannot be said to anticipate the present claims. The rejection over this reference should therefore be withdrawn.

III. CONCLUSION

In conclusion, it is respectfully requested that the above amendments, remarks and requests be considered and entered. Applicant respectfully submits that all the present claims are in compliance with 35 U.S.C. § 102 as being novel over the cited art. The claims are therefore in condition for allowance, and Applicants respectfully requests early notice of such favorable action.

Examiner Lieto is respectfully requested to contact the undersigned at (202) 496-7845 with any questions or comments if they will assist in the understanding this amendment and response. Moreover, if only minor amendments or changes are required to put this case into condition for allowance, the undersigned requests that the Examiner call him to discuss the matter before issuing the next Action.

If these papers are not considered timely filed by the Patent and Trademark Office, then a petition is hereby made under 37 C.F.R. §1.136, and any additional fees required under 37 C.F.R. §1.136 for any necessary extension of time, or any other fees required to complete the filing of this response, may be charged to Deposit Account No. 50-0911. Please credit any overpayment to deposit Account No. 50-0911. A duplicate copy of this sheet is enclosed.

Respectfully submitted,

Date: November 2, 2006

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